REMARKS

Claims 1, 2, 6, 8-12, 14, 17, 19, 21-28, 32-36, 38-45, 83, 84, 86, 87, 89-93 are pending in this application. Applicants have amended claims 1, 6, 14, 17, 19, 38-41, and 84, and canceled claims 3-5, 13, 15, 16, 29-31, 37, 46, 85, and 88 without prejudice or disclaimer.

Rejection of Claims 1-6, 8-13 and 90-93 Under 35 U.S.C. §103(a)

Claims 1-6, 8-13 and 90-91 are rejected under 35 U.S.C. §103(a) as being "unpatentable over Sevarino et al. (Cell, 1989, 57(1):11-19), in view of Stoller et al. (J. Cell Biol., 1989, 108:1647-55 ...), Habener et al. (US 5,118,666), Suzuki et al.(US 5,891,671), and Patel et al. (CIBA Foundation Symposium, 1995, 190:26-50." In addition, the Examiner rejected claims 14, 17, 19, 21-35, 37-46, 83-89 and 92-93 "as being unpatentbale over Sevarino et al. (Cell, 1989, 57(1):11-19), in view of Stoller et al. (J. Cell Biol., 1989, 108:1647-55 ...), Habener et al. (US 5,118,666), Suzuki et al.(US 5,891,671), and Patel et al. (CIBA Foundation Symposium, 1995, 190:26-50), ... and further in view of Warren et al. (Cell, 1984, 39(3 Pt.2):547-55), and Selden et al., U.S. 6,531,124."

Applicants respectfully traverse this rejection for several reasons. First, based on the knowledge in the art at the time of filing, a skilled artisan would not have been motivated to combine the prepro region of somatostatin with GLP-1 and would not have reasonably expected that such a combination would successfully result in expression and secretion of GLP-1. Thus, Applicants still maintain that the PTO has not established a *prima facie* case of obviousness. Furthermore, even if the PTO has established a *prima facia* case of obviousness (which is not the Applicants' position), arguments provided in this reply demonstrate surprising results which are sufficient to rebut any *prima facie* case. These arguments are discussed in detail below.

I. Contrary to the Examiner's assertions, Severino and Stoller do not teach or suggest that the prepro-region of somatostatin would result in secretion of ***any*** heterologous polypeptide.

The Examiner asserts that

> In the instant situation, besides the therapeutic reasons for making GLP-1 taught by Habener, both Sevarino and Stoller teach an expression vector comprising nucleic acid sequences encoding the prepro region of a preprosomatostatin and a heterologous polypeptide, which would allow the target peptide to be secreted, greatly facilitating the purification process. The combination of therapeutic use of GLP-1 and advantages of using an expression vector comprising nucleic acid sequences encoding the prepro region of a somatostatin to make a heterologous polypeptide taught by the cited reference provide strong motivation to make the construct as claimed.

Applicants respectfully disagree with the above-quoted assertion, and in particular to the assertion that Sevarino and Stoller teach that the prepro-region of somatostatin allows secretion of ***any*** heterologous polypeptide. This generalization oversimplifies what Sevarino and Stoller actually disclose.

Sevarino et al. disclose rat somatostatin prepro-region linked to anglerfish somatostatin. Throughout prosecution, the Examiner has maintained that Sevarino teach successful expression of a heterologous peptide by using the prepro-region of somatostatin. See, e.g., page 3, lines 21-24 of the Office Action. While it is true that Sevarino et al. use the prepro region of somatostatin from one species, namely rat, to secret somatostatin from a different species, namely anglerfish, one of ordinary skill in the art at the time of filing would view this reference as teaching that the prepro-region of somatostatin results in secretion of somatostatin and not any peptide as alleged by the Examiner. A comparison of the amino acid sequence of rat somatostatin (14) to anglerfish somatostatin I and anglerfish somatostatin II show that rat and anglerfish somatostatin have identical or nearly identical amino acid sequences. Specifically, the amino acid sequence of rat somatostatin and anglerfish somatostatin I are identical and the amino acid sequences of rat somatostatin and anglerfish somatostatin II differ by only two amino acids (one of which is a conservative substitution). See the attached BLAST alignments submitted herewith as Exhibits A and B. Thus, Sevarino et al. do not disclose that the prepro-region of somatostatin results in secretion of ***any*** peptide as asserted by the Examiner. Instead, because the amino acid sequence of rat and anglerfish mature somatostatin is identical or nearly identical between these two species, Sevarino et al. disclose that the prepro-region of somatostatin results in the secretion of somatostatin.

Stoller et al. disclose secretion of a large, cytoplasmic peptide, α-globin, when linked to an anglerfish somatostatin prepro-region. α-globin is a large protein of 142 amino acids. It was known in the art at the time of filing that expression and secretion of small peptides in active form involve different technical challenges (such as increased degradation) than expression of larger proteins such as α-globin. In addition, Stoller et al. teach that α-globin was used because "it is a cytoplasmic protein and is unlikely to possess intrinsic sorting information." In contrast, GLP-1 is a small, secreted peptide, not a large, cytoplasmic protein. Therefore, Stoller et al. disclose the use of the prepro-region of somatostatin for secretion of large cytoplasmic proteins and not secretion of any heterologous peptide as asserted by the Examiner.

II. Sevarino et al. and Stoller et al. do not provide a complete understanding of the knowledge of those skilled in the art at the time of filing regarding the use of the prepro-region of somatostatin to obtain secretion.

The Examiner maintains that the motivation to combine references is based upon "both Sevarino and Stoller teach[ing] an expression vector comprising nucleic acid sequences encoding the prepro-region of preprosomatostatin and a heterologous polypeptide, which would allow the target peptide to be secreted." The Examiner alleges that the motivation to combine is not based upon hindsight "so long as it takes into account ***only knowledge which was within the level of ordinary skill at the time the claimed invention was made***, and does not include knowledge gleaned only from applicant's disclosure." (***emphasis added***).

However, Sevarino et al. and Stoller et al. do not provide a complete understanding of the knowledge of those skilled in the art at the time of filing regarding the use of the prepro-region of somatostatin to obtain secretion. Applicants submit herewith Danoff et al., 1993, *Eur. J. Biochem.* 218:1063-1070 as Exhibit C. Danoff et al. is from the ***same*** research group which produced the Sevarino et al. and Stoller et al. references. The Danoff et al. reference published ***after*** Sevarino et al. and Stoller et al. and ***before*** the filing date of the present application. Danoff et al. report that despite the fact that the prepro-region of somatostatin provided secretion of α-globin, the prepro-region of somatostatin did ***not*** allow secretion of another protein, chloramphenicol acetyl transferase (CAT). Danoff et al. conclude that the prepro-region of

somatostatin does not work universally and suggest that conformation of the protein to be secreted maybe involved. For example, at page 1069, Danoff et al. provide

> These data imply that even though the somatostatin propeptide is able to confer sorting information to some molecules not normally targeted to the secretory pathway, it cannot function as a universal targeting sequence except when the passenger polypeptide is also able to fold into a stable structure compatible with ER and Golgi transport.

Thus, based upon the state of the art at the time of filing regarding the use of prepro somatostatin, a skilled artisan would not infer that the prepro-region of somatostatin provides secretion of any heterologous polypeptide.

III. None of the remaining references cited by the Examiner would motivate a skilled artisan to use the prepro-region of somatostatin to secret GLP-1.

The remaining references do not make up for the deficiencies of Sevarino et al. and Stoller et al.

Habener discloses that GLP-1 is a potential therapeutic agent for treatment of diabetes and that production of GLP-1 by "conventional means such as by the well-known solid-phase peptide synthesis ... by fragmenting the naturally occurring amino acid sequence or through recombinant DNA technology." Column 4, line 68 through column 5, line 12 of Habener. Habener et al. provide no actual exemplification of how to obtain GLP-1. In addition, Habener make no mention of any signal peptide other than the prepro region provided by glucagon.

Suzuki et al. disclose a method for producing a chimeric protein that can be cleaved and efficiently produced as an inclusion body in *E.coli* under large scale culture. Column 2, lines 6-11 of Suzuki et al. The chimeric protein described by Suzuki includes a protective peptide such as B-galactosidase, a linker and a peptide. As opposed to the claimed vectors and cells, designed to provide expression and secretion of active GLP-1 from a cell, Suzuki et al. teach producing the chimeric proteins in inclusion bodies. Thus, Suzuki et al. do not teach or suggest secretion of a peptide from a cell at all.

Patel et al. discloses the use of pro-protein convertases such as furin, PACE4 and PC1-6 to mediate endoproteolysis of prosomatostatin. Further Patel et al. provide that "PC-12 cells

were similar to COS-7 cells in that they exhibited inefficient constitutive processing of prosomatostatin."

None of these references teach or suggest using the prepro region of somatostatin to obtain efficient secretion of any heterologous protein, much less GLP-1 specifically. Thus, none of these references make up for the deficiencies of Sevarino et al. and Stoller et al.

IV. In view of the state of the art at the time of filing and the references cited by the Examiner, a skilled artisan would not have a reasonable expectation of obtaining efficient secretion of active GLP-1 from a cell using the prepro-region of somatostatin.

The Examiner asserts that

> a prior art reference only needs to provide an indication of success. In the instant case, Sevarino and Stoller have independently demonstrated successful expression of two different heterologous peptides by fusing pro-regions of pro-somatostatin with the target peptide, which would be considered by one of ordinary skill in the art to be sufficient for indicating a reasonable expectation of success for the expression of other heterologous peptides.

Specifically, the Examiner states that

> The key point here is that Sevarino and Stoller successfully and independently used the same system to express distinct peptides, which share neither sequence homology nor molecular size, indicating that neither sequence homologous or size of the molecule to be expressed is critical in this expression system. Given the fact that GLP-1, like somatostatin, is a small peptide hormone, and sequence homology does not seem to play a role as to expression in this system, it is more likely than not that GLP-1 would be expressed successfully in the system in the absence of any indication to the contrary.

Applicants respectfully disagree with this assertion. First, contrary to the Examiner's statements, a skilled artisan reading Sevarino et al. would not view it as demonstrating expression of a "heterologous" peptide. As discussed above, the fact that Sevarino et al. use a prepro-region from rat somatostatin and somatostatin from anglerfish does not indicate that one could successfully express and secrete any small heterologous peptide. The amino acid sequence of rat somatostatin is identical or nearly identical to anglerfish somatostatin. See Exhibits A and B. Therefore, Sevarino et al. demonstrate that the prepro-region of somatostatin can be used to

express and secrete a peptide sequence normally associated with this region. In contrast, a BLAST of the amino acid sequence of human GLP-1 and rat somatostatin revealed no significant sequence homology. See Exhibit D submitted herewith. Thus, a skilled artisan reading Sevarino et al. might expect success in secreting somatostatin with a prepro-region naturally associated with somatostatin but would not reasonably expect successful secretion of a completely different small peptide amino acid sequence with the prepro-region of somatostatin.

Stoller et al. disclose using the prepro-region of somatostatin to obtain secretion of a large, cytoplasmic protein, α-globin. As discussed above, Stoller et al. specifically disclose the selection of α-globin because it is a cytoplasmic protein that does not contain its own sorting information. Moreover, it was known in the art at the time of filing that expression and secretion of small peptides in active form involve different technical challenges (such as increased degradation) than expression of larger proteins such as α-globin. Thus, a skilled artisan would not reasonably expect successful expression and secretion of a small, secreted GLP-1 peptide based on the teachings of Stoller et al.

Furthermore, Sevarino et al. and Stoller et al. do not provide a complete picture of the knowledge in the art at the time of filing. As discussed above, the Danoff et al. reference published after Sevarino et al. and Stoller et al. and is from the same group that published Sevarino et al. and Stoller et al. Danoff et al. report that despite the fact that the prepro-region of somatostatin provided secretion of α-globin, the prepro-region of somatostatin did ***not*** allow secretion of another protein, chloramphenicol acetyl transferase (CAT). Danoff et al. conclude that the prepro-region of somatostatin does not work universally and suggests that conformation of the protein to be secreted may be involved.

Thus, the state of the art at the time of filing demonstrated that one large ***cytoplasmic*** protein was secreted using the prepro-region of somatostatin while a second large ***secreted*** protein was not secreted when the prepro-region of somatostatin is used. In addition, the art had demonstrated that the prepro-region of somatostatin can be used to secrete a peptide naturally associated with that region. It provides no reason to expect that a small, secreted peptide with no significant homology to somatostatin, would be secreted using the prepro-region of somatostatin.

V. With regard to Claims 14, 17, 19, 21-46, 83-89 and 92-93, the additional references cited by the Examiner do not make up for the deficiencies of Sevarino et al. and Stoller et al. discussed above.

As discussed above, none of Sevarino et al., Stoller et al., Habener et al., Suzuki or Patel et al. teach or suggest the claimed invention. None of Warren, Nagai or Selden make up for these deficiencies.

Warren disclose the prepro-region of somatostatin results in low levels of expression of somatostatin in COS cells. Thus, Warren et al. provides absolutely no motivation to use the prepro-region of somatostatin with GLP-1 to obtain expression and secretion of GLP-1 from a cell. Moreover, based upon the disclosure in Warren that the prepro-region of somatostatin only gives low levels of expression of the protein with which it is naturally associated, a skilled artisan would not expect to get efficient expression and secretion of the non-somatostatin small peptide, GLP-1, from a non-endocrine cell using the prepro-region of somatostatin.

Nagai et al. disclose a Factor Xa cleavage site. However, nothing in the Nagai et al. references teaches or suggests use of the prepro-region of somatostatin with any peptide, much less GLP-1.

Furthermore, Selden is not a proper reference to be cited against the present application because the present application was still pending after December 2004 and at the time the claimed invention was made the present application and U.S. Patent Number 6,531,124 "were owned by the same person or under an obligation to assignment to the same person." U.S. Patent Number 6,531,124 was assigned to Transkaryotic Therapies Inc. and recorded at reel 10305/0565 on December 12, 1999. The present application was under obligation to be assigned to Transkaryotic Therapies Inc. as of its filing date and was assigned to Transkaryotic Therapies and recorded at reel 11818, frame 0805 on May 21, 2001.

For the reasons provided above, Applicants submit that the Examiner has not established a *prima facie* case of obviousness against the claimed invention.

VI. Applicants' discovery that GLP-1 can be secreted from non-endocrine cells using the prepro-region of somatostatin at significant levels was unexpected and surprising.

Although Applicants do not concede that the PTO has met its burden of establishing a *prima facie* case of obviousness, the following argument relies on surprising results which are sufficient to rebut any *prima facie* case. The constructs of the invention resulted in surprisingly high levels of secretion from non-endocrine cells.

Warren et al. discuss expression and secretion of somatostatin in COS cells when somatostatin is associated with its naturally occurring prepro region. In that reference, Warren et al. report that using sensitive detection methods such as high performance liquid chromatography (HPLC) relatively low levels of secreted somatostatin can be detected. Warren et al. further provide that somatostatin secretion may not have been detected if SDS-PAGE was used for detection. See page 553, paragraph abridging columns 1 and 2 of Warren et al. Thus, based upon teachings such as Warren et al., it would be expected that even if GLP-1 was secreted by non-endocrine cells when associated with the prepro-region of somatostatin, it would be at fairly low levels.

Further, as provided by the Declaration Under 37 CFR 1.132 of Michael Concino, Ph.D. (hereafter referred to as "the Declaration"), even when GLP-1 was introduced into non-endocrine cells with its naturally associated prepro region, no GLP-1 secretion was detected. The Declaration describes an experiment where a vector containing nucleic acids encoding the signal peptide and propeptide from glucagon as well as GLP-1 was transfected into fibroblasts. Using pulse chase detection and radioimmunoassay (RIA) quantification methods, no secretion of GLP-1 from the fibroblasts was detected. However, when a vector containing nucleic acids encoding the prepro-region of somatostatin as well as GLP-1 was transfected into a fibroblast, GLP-1 was secreted in active form at levels of up to 9.5 ng/1 x 10^6 cells/24 hours as quantified by RIA. This was surprising.

Based upon the knowledge in the art that the prepro-region of somatostatin results in low levels of secretion of somatostatin from non-endocrine cells and the findings that the prepro regions naturally associated with GLP-1 result in no detectable GLP-1 secretion from non-

endocrine cells, it was surprising to find that GLP-1 is expressed at statistically significant levels from non-endocrine cells when it is associated with the prepro-region of somatostatin.

Therefore, even if one were to accept that the Examiner's arguments form the basis of a *prima facia* case of obviousness[1], those arguments do not address or rebut the surprising results seen with the claimed invention—they simply do not suggest the level of secretion observed when the claimed conjugates are introduced into non-endocrine cells.

Enclosed is a check for the Petition for Extension of Time fee. Please apply any other charges or credits to deposit account 06-1050.

Respectfully submitted,

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[1] As provided at pages 9 through 15 of this reply, it is Applicants' position that the PTO has not established that the claimed invention is *prima facie* obvious over the cited references.